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Effect of carbohydrates on the production of thaxtomin A by *Streptomyces acidiscabies*

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Abstract Several *Streptomyces* species cause plant diseases, including *S. scabies*, *S. acidiscabies* and *S. turgidiscabies*, which produce common scab of potato and similar diseases of root crops. These species produce thaxtomins, dipeptide phytotoxins that are responsible for disease symptoms. Thaxtomins are produced in vivo on diseased potato tissue and in vitro in oat-based culture media, but the regulation of thaxtomin biosynthesis is not understood. *S. acidiscabies* was grown in a variety of media to assess the impact of medium components on thaxtomin A (ThxA) production. ThxA biosynthesis was not correlated with bacterial biomass, nor was it stimulated by α-solanine or α-chaconine, the two most prevalent potato glycoalkaloids. ThxA production was stimulated by oat bran broth, even after exhaustive extraction, suggesting that specific carbohydrates may influence ThxA biosynthesis. Oat bran contains high levels of xylans and glucans, and both of these carbohydrates, as well as xylans from wheat and tamarind, stimulated ThxA production, but not to the same extent as oat bran. Starches and simple sugars did not induce ThxA production. The data indicate that complex carbohydrates may act as environmental signals to plant pathogenic *Streptomyces*, allowing production of thaxtomin and enabling bacteria to colonize its host.

Keywords *S. acidiscabies* · Plant pathogenic *Streptomyces* · Thaxtomin · Cell wall carbohydrate · Xylan · Phytotoxin

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Introduction

A daunting obstacle in the discovery and study of potentially novel compounds is the difficulty in reliably producing secondary metabolites in vitro (Zazopoulous et al. 2003). Microbial genomics has revealed the genes and biochemical pathways behind the synthesis of many secondary metabolites, as well as many putative biosynthetic genes and gene clusters with which no compounds have been associated (Donadio et al. 2002; Thompson et al. 2002). The genome of *Streptomyces avermitilis* contains five type-I polyketide synthase gene clusters with no identified products (Omura et al. 2001; Ikeda et al. 2003). Similarly, the genome of *S. coelicolor* contains many gene clusters that are predicted to code for secondary metabolites (Bentley et al. 2002). The fact that the genome of *S. coelicolor* encodes for 65 sigma factors and 965 proteins with predicted regulatory functions indicates that some pathways resulting in secondary metabolite biosynthesis may be tightly regulated (Bentley et al. 2002).

Forty-five of the sigma factors in *S. coelicolor* are involved in the transcription of genes in response to environmental stimuli, suggesting that to produce some secondary metabolites in vitro, it may be necessary to grow the microorganism under specialized conditions while supplying a specific elicitor.

One kind of relationship in which secondary metabolites often play a key role is that of disease. Common scab of potato is a bacterial disease in which phytotoxic secondary metabolites, called thaxtomins, cause corky lesions to form on the periderm of immature potato tubers. The lesions enlarge and coalesce as the tuber grows, resulting in poor consumer acceptance, increased costs to potato processors, and significant losses to potato growers (Loria et al. 1997, 2003). Three species of *Streptomyces*, *S. scabies*, *S. acidiscabies*, and *S. turgidiscabies*, produce thaxtomins and cause common scab of potato (King et al. 1992; Loria et al. 1997). Thaxtomins are essential for the development of scab symptoms (Lawrence et al. 1990; Healy et al. 2000) and appear to disrupt the synthesis of cellulose by plant cells (Lawrence et al. 1990; Fry and Loria 2002). Thaxtomins are cyclic dipeptides consisting of one molecule of tryptophan and one molecule of phenylalanine, which are joined by a bimodular peptide synthetase, designated *txtA* and *txtB*. Individual thaxtomins are distinguished by the presence of methyl groups attached by *txtA* and *txtB* (Healy et al. 2000) and by hydroxyl groups attached to the dipeptide backbone by a P450 monooxygenase, *txtC* (Healy et al. 2002). All thaxtomins possess a nitro group at the 4-position on the indole ring of the tryptophan moiety mediated by the action of a nitric oxide synthase (NOS)(Kers et al. 2004), which is necessary for their phytotoxicity (King et al. 1992). The genes for the production of thaxtomins are present in all three of the scab-causing species and are borne on a mobile pathogenicity island (Healy et al. 2000; Bukhalid et al. 2002).

All three scab-producing *Streptomyces* species produce and secrete thaxtomins, predominantly thaxtomin A (ThxA), in infected tissues and, under the proper culture conditions, in vitro (Babcock et al. 1993; Loria et al. 1995; King and Lawrence 1996a), but the regulation of ThxA production is not understood. There is evidence that the bacteria produce ThxA in response to environmental cues. For example, *Streptomyces* will not produce ThxA in common bacterial media, such as Luria broth and tryptic soy broth (Loria et al. 1995), but a medium consisting solely of oatmeal or oat bran and water supports the production of ThxA (Babcock et al. 1993; King and Lawrence 1996a). Maximal ThxA biosynthesis does not occur until several days after culture inoculation (Loria et al. 1995; King and Lawrence 1996b), indicating that developmental regulation also plays a role.

Although there are no reports of oat diseases caused by *Streptomycetes*, scab-causing *Streptomyces* species have a wide host range and the response to ThxA is conserved in monocots and dicots even though the specific target has yet to be identified (Fry and Loria 2002). Identifying the conditions under which ThxA production in vitro occurs may facilitate identification of the environmental cues that trigger ThxA production in vivo. This knowledge may suggest cultural conditions for potatoes that reduce disease incidence or a potential target for breeding scab resistance since no cultivar is fully resistant (Tai et al. 1996; Hiltunen et al. 2005; Pasco et al. 2005). In addition, understanding the regulation of ThxA biosynthesis may provide valuable insights into the factors that stimulate the production of secondary metabolites in other microorganisms.

Materials and methods

Materials

Tamarind xyloglucan (Megazyme, Bray, Republic of Ireland), wheat arabinoxylan (Megazyme), and β-1-4-xylan were kindly provided by Jocelyn Rose, Plant Biology, Cornell University. All other reagents were obtained from Sigma (St Louis, MO). Oat bran was obtained from New Hope Mills (Moravia, NY).

Bacterial strains, media, and culture conditions

Streptomyces acidiscabies strain 84.104 was used in all assays. Media treatments included oat bran broth (OBB) (Shirling and Gottlieb 1966), CRM (1% glucose, 10.3% sucrose, 1.5% tryptic soy broth, 0.5% yeast extract, 50 mM $MgCl_2$) and YEME (1% glucose, 0.5% Bacto-peptone, 0.3% malt extract, 0.3% yeast extract, 10% sucrose, 5 mM $MgCl_2$). Potato tuber medium (PTM) was prepared from immature potato (*Solanum tuberosum*, cv. "Chippewa") tubers, less than 1 cm in diameter. Tubers produced from stem cuttings (Healy et al. 2002) were freeze-dried and ground to a powder. PTM contained 20 g/l of the powdered tubers and 2 ml/l of trace element solution (Kieser et al. 2000). For some experiments, 25% OBB was prepared by diluting OBB 1:3 with deionized water. For comparisons of effects due to carbohydrate source, media were prepared containing either 2 g/l ammonium sulfate or 1 g/l yeast extract as a nitrogen source and 7 g/l of carbohydrate and 2 ml/l of trace element solution. All media were adjusted to pH 7.2. For spore production,

bacteria were grown on solid ISP4 medium (Becton, Dickinson; Sparks, MD) at 28–30°C for 5–7 days; spores were scraped from the substrate mycelia and stored at −80°C, in 20% glycerol.

Thaxtomin extraction and analysis

Cultures were harvested by filtration through pre-weighed Miracloth™ to collect bacterial mycelia, which were subsequently dried and weighed. Dry weights could not be determined accurately when bacteria were grown with cellulose as a carbon source, because cellulose particles collected on the Miracloth along with the bacterial mycelia; therefore only total ThxA yields are reported for the carbohydrate studies. Culture filtrates (4 ml) were passed through Alltech (Deerfield, IL) Extract-Clean solid phase extraction cartridges (C18; 200 mg), rinsed with deionized water, then with methanol:water (25:75), and ThxA was eluted with methanol:water (50:50). ThxA was analyzed using HPLC as described previously (Healy et al. 2000), but using an isocratic mobile phase of acetonitrile:water:trifluoracetic acid (40:60:0.1). ThxA was detected via absorbance at 215 and 380 nm and quantified using pure ThxA as a standard. The limit of detection for Thaxtomin A was 10 ng, with a signal to noise ratio of 25.

Extraction of oat bran and bioassay of fractions

Oat bran (100 g) was first extracted with 300 ml of hexane for 30 min at room temperature. The hexane was removed via vacuum filtration, evaporated to dryness, and weighed. After air-drying, the bran was then sequentially extracted in a similar fashion with 300 ml ethyl acetate, methanol, and water. The water extract and the residual bran were freeze-dried and weighed. The masses of the hexane, ethyl acetate, methanol, and water extracts were 4.68, 1.03, 0.53, and 1.96 g, respectively. Bacteria were then grown in 25% OBB to which one extract was added using a concentration of the extract equivalent to its concentration in 100% OBB. Growing the bacteria in 25% OBB (allowing a baseline level of Thx production relative to 100% OBB) was necessary in order to evaluate comparative levels of Thx production among treatments. To assay the extracted bran residue, OBB medium was made with the residue, using 20 g/l. The resulting broth was mixed 3:1 with normal OBB.

Treatment of oat spelt xylans with xylanase

To determine the effect of xylan fragmentation on bacterial growth and ThxA production, medium was prepared containing 7 g/l oat spelt xylans, 2 g/l ammonium sulfate, 2 ml/l trace element solution, and adjusted to pH 4.5. The medium was divided into 50 ml aliquots, then 5 units of endoxylanase (from *Trichoderma viride*-capable of releasing 5 μmol xylose equivalents per minute via cleavage of internal linkages), was added to each aliquot, and the medium was incubated at 30°C for 0, 1, 2, 4, and 24 h. Enzymatic digestion was stopped by adjusting the medium pH to 7.2 followed by autoclaving for 18 min.

Statistical analysis

Experiments were analyzed using the JMP® statistical software 4.0.3 version. Treatments were compared to controls with ANOVA statistical analysis using Tukey's HSD test ($P < 0.05$) and reported as mean ± standard error of the mean (SEM).

Thaxtomin production assays

Six-well plates were prepared with 4 ml of medium per well, then inoculated with approximately 5×10^6 spores of 84.104, and shaken at 150 rpm, 25–28°C. After 3 days, the cultures were harvested, biomass dry weights were determined, and ThxA production was measured as described above. Treatments were replicated three to six times, and experiments were independently replicated at least twice under identical growing conditions.

Results

Relationship between bacterial growth and ThxA production

S. acidiscabies strain 84.104 was grown on four different media: OBB, CRM, YEME, and PTM. All the media supported growth of 84.104, but ThxA was detected only in the two media made directly from plant tissues: OBB and PTM which was less than 5% of OBB prduction (Table 1). The data indicate that there is no apparent correlation between bacterial growth and ThxA production and suggest that ThxA production may be stimulated by the presence of substances derived from plant tissues.

Effect of solanine and chaconine on ThxA production

Potato tubers contain steroidal glycoalkaloids, mainly α-solanine and α-chaconine, that are concentrated in the tuber periderm, where total glycoalkaloid content

Table 1 Thaxtomin production and dry weights of samples grown in presence of various media components

Media treatment	Thaxtomin produced as percentage control in OBB	Dry weight of biomass produced as percentage of control in OBB
OBB (control)	100.00 (3.10) a	100.00 (3.11) b
CRM	0.00 (0.00) b	177.75 (7.27) a
YEME	0.00 (0.00) b	68.02 (5.91) c
PTM	2.10 (0.36) b	54.76 (0.99) c
OBB with the addition of:		
Solanine (1 μg)	99.35 (1.88) a	101.18 (2.57) a
Solanine (10 μg)	92.40 (1.94) ab	103.14 (3.72) a
Solanine (50 μg)	84.94(2.07) bc	102.95 (2.48) a
Chaconine (1 μg)	99.67 (2.94) ab	99.21 (3.38) a
Chaconine (10 μg)	95.32 (2.96) b	105.89 (2.94) a
Chaconine (50 μg)	71.82 (0.73) c	110.61 (2.54) a
OBB	100.00 (0.42) a	100.00 (1.35) a
YE	7.42 (1.47) b	6.29 (0.45) e
YE + xylan	1.60 (0.12) b	51.53 (1.06) b
YE + starch	1.55 (1.11) b	22.11 (0.61) d
YE + sucrose	1.56 (0.49) b	31.80 (0.34) c

Quantitative estimates are expressed as percentage of control values using OBB as the control (mean ± SEM, $n = 6$). Means within a column not followed by the same letter are significantly different at $P < 0.05$ in Tukey's HSD test

can be as high as 0.1% on a dry weight basis. Potato alkaloids are known to inhibit the growth of fungi and may have a role in fungal disease resistance (Lisinska and Leszczynski 1989). When 84.104 was grown in OBB containing α-solanine or α-chaconine at 0.25, 2.5, or 12.5 μg/ml, no stimulation of ThxA production was found (Table 1). ThxA production was inhibited at the highest level of both alkaloids tested (12.5 μg/ml), with no decrease in bacterial growth.

Effect of oat bran extracts on ThxA production

Oat bran was fractionated into several components using sequential solvent extractions, then evaluated for their effects on ThxA production. The hexane, ethyl acetate, and methanol extracts supported bacterial growth but decreased the amount of ThxA produced relative to the OBB control (Fig. 1). The cold water extract stimulated the production of ThxA by a factor of five in comparison to OBB. The extracted residue increased bacterial growth and stimulated production of ThxA by a factor of ten. Others have suggested that potato suberin is an elicitor of thaxtomin production (Beausejour et al. 1999); however, any oat suberin monomers or precursors present in the organic fractions (Bernards 2002) did not support ThxA production. In addition, the PTM media (Table 1), which should contain suberin, only produced low levels of

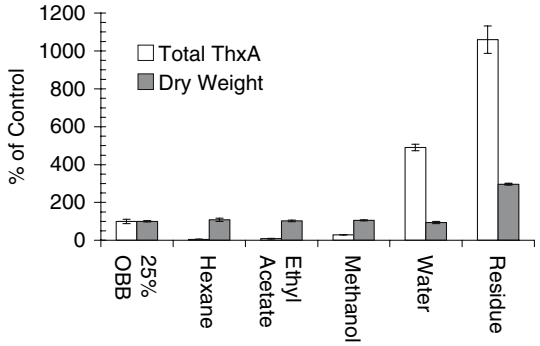

Fig. 1 Effect of fractionated oat bran extracts on total ThxA production and biomass growth of *S. acidiscabies* 84.104, expressed relative to growth and production in 25% OBB (oat bran broth) (mean ± SE)

ThxA, and oat media would be expected to be much lower in suberized tissues (Bunzel et al. 2004). The factors involved in stimulating ThxA production present in oat bran appear to be polar molecules, at least one of which is water-soluble.

Effect of oat xylans on ThxA production

Oat grains contain approximately 60% xylans, specifically arabinoxylan (Miller 1958; Luhaloo et al. 1998). Because xylans are insoluble in hexane, ethyl acetate, and methanol, and only partially soluble in water, we hypothesized that xylans might be one of the components of the solvent-extracted bran that enhanced growth and ThxA production.

We grew 84.104 in media containing yeast extract alone, or with either oat spelt xylan (86% xylose, 8% arabinose, 4% glucose), starch, or sucrose as a carbon source. Bacterial growth in all four media was much lower than in OBB. Strain 84.104 produced measurable amounts of ThxA in all four media tested although at levels much lower than in OBB (Table 1). The xylan-based medium supported higher levels of ThxA production and bacterial growth, but it was not statistically better than YE amended with starch or sucrose alone.

ThxA production in various carbohydrates

The oat bran fractionation results suggested that oat bran contains both water-soluble and water-insoluble factors that stimulate thaxtomin biosynthesis, so we tested the effect of both water soluble, miscible, and insoluble carbohydrates. The polymers-amylose, amylopectin, arabinogalactan, lichenan, potato starch, wheat pentosans, xylan, and xyloglucan-swell in or are partially soluble in water, while cellulose is insoluble. In addition to oat spelt xylans, we tested lichenan, a

(1→3), (1→4)-β-D-glucan soluble in boiling water, because oat bran contains from 2–8% of (1→3), (1→4)-β-D-glucan (Luhaloo et al. 1998; Roubroeks et al. 2001). Oat spelt xylans consist of xylose, arabinose, and glucose, so each of these water-soluble sugars was tested individually, in addition to sucrose and cellobiose. In addition, to eliminate any complications due to glucans present in yeast extract (Otero et al. 1996; Koleva et al. 1997–1998), the basal media was reformulated using ammonium sulfate as a nitrogen source.

Bacterial growth on the various carbohydrates varied widely, with the most growth observed on pure amylose (Table 2); however, amylose did not support any ThxA production. Cellobiose and lichenan supported very low levels of bacterial growth but high levels of ThxA production. Xylose, constituting 86% of the monomeric content of oat bran xylan, was the only simple sugar tested that supported a low level of ThxA production. ThxA production on glucose was below detection limits, and strain 84.104 was unable to grow on a medium containing arabinose as the only carbohydrate source (data not shown). Three additional xylans were tested, including β-1,4 xylan composed solely of xylose; wheat pentosan, which is 37% arabinose, 61% xylose, and 2% other sugars; and tamarind xyloglucan, which is 35% xylose, 45% glucose, 16% galactose, and 4% arabinose. The media that produced the highest levels of bacterial growth and supported the most ThxA production were wheat pentosan, β-1,4 xylan, and lichenan. ThxA production on cellobiose was the highest on a per mg biomass but growth was poor, which may have overestimated yield on a weight basis (Table 2).

Lastly, a medium (SLX) was prepared using 7 g/l each of starch, lichenan, and oat spelt xylan as carbon sources, and compared to media containing the three carbohydrates individually as well as to OBB. Although bacterial growth was higher in SLX than in the media containing only a single carbon source, ThxA production was not significantly different than with xylan or lichenan alone on a percentage basis (Table 2).

Effect of xylan fragmentation

Xylan molecules range in size from 70 to 200 xylopyranose units (Tuncer and Ball 2003). To determine whether xylan polymer size could influence bacterial growth or ThxA production, we digested oat spelt xylans with xylanase for 0, 1, 2, 4, and 24 h and prepared culture media from these digests. Bacterial growth and ThxA production increased with longer xylanase treatments up to four hours, but decreased when treated for 24 h (Table 2).

Discussion

Without the production of thaxtomins, plant pathogenic *Streptomyces* cannot induce the symptoms of

Table 2 Thaxtomin production and dry biomass weights of samples grown in presence of various carbohydrate sources

Media treatment	Thaxtomin produced as percentage OBB production	Dry weight of biomass as percentage of OBB production	μg Thaxtomin per mg biomass
OBB	100.00 (1.20) a	100.00 (11.20) a	11.92 (0.53) b
Amylopectin	0.00 (1.69) c	3.09 (15.84) b	0 d
Amylose	0.00 (1.69) c	52.22 (15.84) ab	0 d
Arabinogalactan	0.00 (1.69) c	3.29 (15.84) b	0 d
Cellobiose	10.44 (1.20) b	2.06 (11.20) b	56.58 (15.62) a
Cellulose	2.32 (1.72) c	78.23 (11.20) a	0.16 (0.02) c
Glucose	0.00 (1.69) c	2.32 (15.84) b	0 d
Lichenan	10.78 (1.20) b	5.52 (11.20) b	13.83 (5.69) b
Starch	0.00 (1.69) c	3.09 (15.84) b	0 d
Sucrose	0.00 (1.69) c	3.87 (15.84) b	0 d
Tamarind xyloglucan	0.98 (1.69) c	2.02 (15.84) b	8.56 (4.76) bc
Wheat pentosans	7.83 (1.69) bc	12.32 (15.84) b	7.06 (1.34) bc
Oat spelt xylan	3.63 (1.21) c	12.00 (15.84) b	0.24 (0.02) c
β 1,4 xylan	8.77 (1.72) b	7.47 (15.84) b	13.17 (1.85) bc
Xylose	0.65 (0.56) c	2.71 (15.84) b	2.90 (1.45) c
SLX	15.31 (0.41) b	29.80 (2.96) ab	5.28 (0.22) c
Xylan with xylanase, 0 h	3.04 (0.35) b	13.28 (0.46) b	2.02 (0.28) c
Xylan with xylanase, 1 h	3.91 (0.03) b	14.83 (1.24) ab	2.35 (0.21) c
Xylan with xylanase, 2 h	4.03 (2.01) b	12.76 (1.53) b	2.50 (1.25) c
Xylan with xylanase, 4 h	9.13 (0.16) a	19.48 (0.76) a	3.99 (0.02) b
Xylan with xylanase, 24 h	8.59 (0.36) a	12.07 (1.38) b	6.50 (1.13) b

Quantitative estimates are expressed as μg total thaxtomin produced in the sample treatment as a percentage of total thaxtomin production in OBB; data are expressed as the mean (SEM, $n = 6$). Means within a column not followed by the same letter are significantly different at $P < 0.05$ in Tukey's HSD test

common scab of potato, so understanding the circumstances under which thaxtomins are made is key to understanding the disease process. Plant pathogenic *Streptomycetes* are known to synthesize thaxtomins under two seemingly unrelated circumstances: in vivo within the periderm of infected potato tubers and in vitro in oat-based culture media. We have presented data here indicating these two different environments may send analogous signals to the bacterium, which result in thaxtomin production.

The potato tuber periderm is notably high in alkaloids, and could potentially act as a signal for plant host colonization, resulting in an increase in bacterial growth rate and/or an increase in the production of thaxtomins. However, we found that the two most prevalent potato alkaloids, α-solanine and α-chaconine, do not stimulate bacterial growth. When present at levels five times that found in the periderm, the alkaloids decreased ThxA production. Potatoes are also particularly high in starch, but when *S. acidiscabies* was cultured on potato starch, amylose or amylopectin, the media supported bacterial growth, but no ThxA production. The sugar content of potato tubers can be as high as 10% of the dry weight (Kadam et al. 1991), and it has been reported that the incidence of potato scab is correlated with the level of reducing sugars, specifically glucose, in the tuber (Goto 1985). However, we did not find appreciable ThxA production on glucose, and when oatmeal broth is supplemented with glucose, thaxtomin production is inhibited (Loria et al. 1995).

In addition to starch, oats have high levels of two other carbohydrates: xylans and glucans. When cultured on xylans and glucans, *S. acidiscabies* was able to produce ThxA, even when bacterial growth was poor. For example, if calculated on a per dry weight biomass basis, the ThxA production on lichenan was over ten times what was found in oat bran broth. On the other hand, the monomer components of these polymers supported little or no ThxA production, indicating that the polymers or their oligomeric breakdown products are specifically recognized as cues to initiate thaxtomin biosynthesis.

None of the individual components of oat bran supported levels of bacterial growth and ThxA production equivalent to those supported by oat bran broth. In creating defined media to compare individual carbohydrates, we limited the growth of the bacterium and presumably ThxA production as well. When oat spelt xylan and lichenan were combined with starch in a single medium (SLX), bacterial growth was increased, but ThxA production was not significantly greater than with a medium containing only xylan. However, we have demonstrated that a high level of bacterial growth does not necessarily lead to thaxtomin production, since there are several culture media on which all three species will grow rapidly, but will not produce any thaxtomins. The data therefore indicate that thaxtomin biosynthesis is not linked to bacterial growth, and it is possible that once thaxtomin production is initiated, additional nutrients that support rapid bacterial growth and metabolism may result in higher levels of thaxtomin production.

Streptomycetes are capable of utilizing a wide variety of carbohydrates and other polymers not exploited by other soil microorganisms (Madigan et al. 2000; Loria et al. 2003), due to a repertoire of highly regulated, secreted enzymes such as cellulases and xylanases that digest these large molecules into monomers and small oligomers, such as cellobiose, a product of cellulose degradation (Madigan et al. 2000). *Streptomycetes* are known to possess a cellobiose ABC-type transporter that brings external cellobiose inside the cell (Fernandez-Abalos et al. 1997), that, in turn, stimulates the production and secretion of cellulases. Similarly, the production of Streptomycete xylanases is elicited by the presence of xylans (López Fernåndez et al. 1995; Antonopoulos et al. 2001).

In this study, the growth of strain 84.104 on xylans that have been partially fragmented by xylanase resulted in higher levels of ThxA production, when compared with intact xylan. Common scab infections are initiated on immature potato tubers in which the primary cell walls contain high levels of xylans. We have demonstrated that xylans can support thaxtomin production, and that starch, abundant in tubers, supports bacterial growth. When a potato tuber supplies these two components, the bacterium may respond by producing ThxA and, ultimately, disease symptoms. Oats, coincidentally, seem to contain an optimal combination, lacking in potatoes, that makes oat bran a superior medium for bacterial growth and ThxA production. Alternatively, thaxtomin production and bacterial growth that result in host colonization may be not be due solely to host-supplied signals, but might also depend on the soil environment.

None of the signals that we found to support thaxtomin production are unique to potatoes. Starch, glucans, and xylans are common plant carbohydrates, and are present in most roots, and because thaxtomins seem to target cellulose biosynthesis, one would expect plant pathogenic *Streptomycetes* to have broad host ranges. In fact, these organisms can infect underground structures of many crops, and are neither host nor tissue specific (Loria et al. 1997, 2003). Interestingly, we found that a medium made solely from immature potato tubers, the specific tissue colonized

by pathogenic *Streptomycetes*, supports considerably less biomass and ThxA production than an oat bran-based medium. The crux of the disease relationship may therefore lie not in the mere stimulation of thaxtomin production nor in the amount of thaxtomin produced by the pathogen. Instead, common scab may be the result of the length of time over which tubers expand during which time expanding cell walls are available for infection. Our work with plant pathogenic *Streptomyces* provides evidence that specific carbohydrates may provide signals that support secondary metabolite pathways required for pathogenicity.

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